In re Application of: Gil Ronen et al.
Serial No.:10/561,220
Filed: April 4, 2006
Office Action Mailing Date: January 30, 2008

Examiner: WORLEY, Cathy Kingdon
Group Art Unit: 1638
Attorney Docket: 30698

In the Specification:

Please amend the Paragraph beginning at **Page 17, line 27**, as follows:

Down-regulation of such trichome components may be effected by down-regulating genes which are involved in the production or accumulation of these components. For example, gene products which are involved in exudate synthesis may be revealed by genome and EST mining and directed gene knock-out. Gene mining includes the identification in public databases (e.g., GenBank ~~www~~ World Wide Web (dot) ~~-~~ncbi (dot) ~~-~~nlm~~-~~ (dot) nih (dot)~~-~~ gov/Genbank/index/html) of ortholohgous sequences deriving from the plant of interest which share homology with known genes in the pathway using sequence alignment software such as BLAST (~~www-~~ World Wide Web (dot) ncbi~~-~~ (dot) nlm (dot) ~~-~~nih (dot)~~-~~ gov/BLAST). Alternatively, trichome EST libraries may be useful for identifying genes which are involved in metabolite synthesis [see for example Lange (2000) Proc. Natl. Acad. Sci. 97:2934-2939; Gang (2001) Plant Physiology 125:539-555].

Please amend the Paragraph beginning at **Page 19, line 3**, as follows:

Synthesis of RNAi molecules suitable for use with the present invention can be effected as follows. First, the mRNA target sequence is scanned downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein

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siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level (World Wide Web (dot) ~~www.~~ambion (dot) ~~.~~com/techlib/tn/91/912 (dot) ~~.~~html).

Second, potential target sites are compared to an appropriate genomic database (e.g., human, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server (World Wide Web (dot) ~~www.~~ncbi (dot) ~~.~~nlm~~.~~ (dot) nih (dot)~~.~~ gov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

Please amend the Paragraph beginning at **Page 19, line 29**, as follows:

Antisense and siRNA technology has been used in selective downregulation of two tobacco trichome genes encoding different enzymes [Wang (2002); J. of Exp. Bot. 53:1891-1897; Wang (2003) Planta 216:686-691]. siRNA oligonucleotides for downregulating PPO for example, may be generated by inserting the cDNA sequence of PPO (GenBank Accession No: Z12833 for PPOA, GenBank Accession No. Z12836 for PPOD) to an siRNA selection software such as provided by World Wide Web (dot) ~~www.~~ ambion (dot) ~~.~~com.

Please amend the Paragraph beginning at **Page 29, line 4**, as follows:

LEADS™ software (Compugen, IL) was used for clustering and assembling the tomato sequences and provided more than 20,000 clusters representing different genes. An expression profile annotative summary was designed for each cluster by pooling all keywords of each sequence represented in the cluster. Clusters were selected based on trichome EST number and percentage out of total ESTs present in each cluster. Clusters were analyzed for ORFs using Vector NTI suite (InforMax, U.K.) version 6. ORFs of each gene were compared to Genbank database, using Blast (~~http~~ Hypertext Transfer

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Protocol://~~www.~~ World Wide Web (dot) ncbi (dot)~~.~~ nlm (dot) ~~.~~nih (dot) ~~.~~gov/BLAST/) and for the highest homologous ORF the position of the ATG start codon and stop codon was compared. Accordingly, most of the sequences described herein were predicted to posses the full length ORF. Clusters were classified as trichome-specific (i.e. more than 90% of ESTs in a cluster were originated from trichome cDNA libraries) or trichome expressed (i.e. at least one of the ESTs in a cluster was originated from trichome cDNA libraries).

Please amend the Paragraph beginning at **Page 31, line 4**, as follows:

In order to clone these promoter sequences and 5' untranslated region (5' UTR) upstream of the ATG starting codon, total genomic DNA was extracted from plant leaf tissues of 4 week old plants of the following species: cultivated tomato (*Lycopersicon esculentum*, var 870), wild tomato species (*Lycopersicon hirsutm,* var LA 1777 and *Lycopersicon pennellii*, var LA 716), tobacco (*Nicotiana tabaccum*, var NN) or cotton (*Gossypium hirsutum* var Acala 23). DNA extraction was effected using DNA extraction kit (~~Dneasy~~ DNEASY plant mini kit, Qiagen, Germany). Inverse PCR (IPCR), DNA digestion, self-ligation, and PCR reaction were performed on genomic DNA, following a well established protocol (~~http~~ Hypertext Transfer Protocol ://~~www.~~ World Wide Web (dot) pmci~~.~~ (dot) unimelb (dot)~~.~~ edu (dot)~~.~~ au/core facilities/manual/mb390.asp) with the following modifications. To avoid mistakes in the IPCR, first the genomic sequence of the 5' sequence of a relevant cDNA (i.e. including introns) was identified to produce Genomic Island (GI). The desired region from the genomic DNA was PCR-amplified using direct oligonucleotide primers designed based on the cDNA cluster sequence, as was predicted by the Leads software (Compugen, IL). PCR reaction was performed in a DNA thermal cycler, using common PCR conditions (for example: 92 °C/3 min followed by 31 cycles × [94 °C/30 sec; 56 °C/30 sec; 72 °C/*3* min] followed by 72 °C/10 min). PCR products were purified using

PCR purification kit (Qiagen) and sequencing of the amplified PCR products was performed, using ABI 377 sequencer (Amersham Biosciences Inc).

Please amend the Paragraph beginning at **Page 39, line 8**, as follows:

Cloning of human growth hormone into a binary vector - The mature polypeptide of the Human-growth hormone gene (HGH, GenBank Accession No: V00519) was produced synthetically using GeneArt service (Hypertext Transfer Protocol~~http~~://World Wide Web (dot) ~~www.~~geneart~~.~~ (dot) de/). The sequence was adjusted according to the tomato codon usage, while avoiding, as much as possible, high GC content and low complexity of DNA sequences. An ATG was added as a first codon to the mature polypeptide enabling sufficient translation. The restriction sites of *Sma*I and *Sac*I were added to the gene at the 5' prime end and 3' prime end, respectively. The sequence of the HGH gene is set forth in SEQ ID NO: 58.

Please amend the Paragraph beginning at **Page 9, line 13**, as follows:

FIG. 1a is a prior art schematic illustration of various tomato trichomes. Type VI glandular trichomes naturally accumulate high levels of the PPO enzyme (Luckwill LC. 1943. The Aberden University Press, Aberden, Scotland).

FIGs. 1b-h are photomicrographs depicting trichome specific expression of GUS under the regulation of the CaMV 35S, TR2, TR5, TR11, TR25 or TR27P promoters. Figure 1b - Trichomes of wild-type tomato plants. Figure 1c - Trichomes of tomato plants overexpressing GUS under the constitutive CaMV 35S promoter. Figures 1d ~~b-h~~ - Trichomes of tomato plants overexpressing GUS under the TR2 promoter. Figure 1e - Trichomes of tomato plants overexpressing GUS under the TR5 promoter. Figure 1f - Trichomes of tomato plants overexpressing GUS under the TR11 promoter. Figure 1g - Trichomes of tomato plants overexpressing GUS under the TR25 promoter. Figure 1h -

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Trichomes of tomato plants overexpressing GUS under the TR27 promoter. ~~Trichomes of tomato plants overexpressing GUS with different trichome promoters~~

Please amend the Paragraph beginning at **Page 9, line 27**, as follows:

FIG~~s~~. 4a~~-e~~ schematically illustrate~~s~~ ~~various embodiments of~~ a trichome mechanical harvester constructed in accordance with some embodiments of ~~the teachings of~~ the present invention.

FIG. 4b schematically illustrates a trichome mechanical harvester constructed in accordance with some embodiments of the present invention.

FIG. 4c schematically illustrates a trichome mechanical harvester constructed in accordance with some embodiments of the present invention.

Please amend the Paragraph beginning at **Page 10, line 6**, as follows:

FIGs. 6a-d are graphs depicting expression levels of three trichome-expressed genes (TR2H, TR4H and TR5H) as determined by RT-PCR. Expression is shown as fold increase over house-keeping gene expression. 273_1 is *L.hirsutum* var glabratum cultivar; 247 is *L. esculentum* cultivar. Figure 6a – a histogram depicting fold increase in the expression level of TR2H in 273_1 and 247 plants as compared to the expression level in house-keeping genes; Figure 6b - a histogram depicting fold increase in the expression level of TR2H in 247 plants as compared to the expression level in house-keeping genes; Figure 6c - a histogram depicting fold increase in the expression level of TR4H in 273_1 and 247 plants as compared to the expression level in house-keeping genes; Figure 6d - a histogram depicting fold increase in the expression level of TR5H in 273_1 and 247 plants as compared to the expression level in house-keeping genes; Tissue key: L- Leaves; L-T- Leaves minus Trichomes; T1 and T2 are two independent RNA samples of Trichome cells.

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Please amend the Paragraph beginning at **Page 29, line 22**, as follows:

Trichome cells were harvested from tomato mature leaves by first freezing the leaves, just above liquid nitrogen and then brushing both sides of the leaves with paint brush, previously chilled in liquid nitrogen. Total RNA was extracted from leaves, trichome cells or leaves minus trichome cells of tomato using ~~Rneasy~~ RNEASY plant mini kit (Qiagen, Germany) using the protocol provided by the manufacturer. Reverse transcription was performed using 1.5 μg total RNA, using 300 U Super Script II Reverse Transcriptase enzyme (Invitrogen), 225 ng random deoxynucleotide hexamers (Invitrogen), 500 μM dNTPs mix (Takara, Japan), 0.2 volume of x5 RT buffer (Invitrogen), 0.01M DTT, 60U ~~RNAsin~~ RNASIN (Promega), DEPC treated DDW was added up to 37.5 μl.